Endobenthic predation by the nemertean *Cerebratulus lacteus* in soft-shell clam (*Mya arenaria*) populations in Prince Edward Island

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Abstract

The milky ribbon worm, *Cerebratulus lacteus* (Nemertinea: Heteronemertini), has been identified as an important threat to soft-shell clam (*Mya arenaria*) populations in Atlantic Canada. The biology of this species, particularly its predatory behavior, is still largely unknown. Field and laboratory studies were undertaken in Prince Edward Island, Canada, to better describe the relationship between *C. lacteus* and *M. arenaria* and determine the feasibility of control tools to reduce predation on soft-shell clam industry production. In the field, density and predation rate of *C. lacteus* were evaluated in relation to sediment manipulation: i) addition of shells and ii) use of a hydraulic rake. These were ineffective for reducing predation of soft-shell clams. In the laboratory, *C. lacteus* was confirmed as an efficient predator of *M. arenaria*. Analysis of clam size selection revealed no significant preference. A complementary set of experiments carried out to see if the sympatric polychaete *Nereis virens* would have any impact on the relationship between *C. lacteus* and *M. arenaria*, revealed no impact on *C. lacteus* predation on clams. Conversely, *N. virens* demonstrated a negative response to the presence of *C. lacteus*.

Résumé

Le némerte *Cerebratulus lacteus* (Nemertinea: Heteronemertini) est identifié comme une menace importante pour les populations de myes (*Mya arenaria*) dans l'est du Canada. La biologie de ce vers, particulièrement son comportement de prédation, est cependant peu connue. Des travaux de terrain et de laboratoire furent entrepris à l'Île-du-Prince-Édouard, Canada, afin de documenter les interactions entre *C. lacteus* et *M. arenaria*. Une plus grande connaissance de ces interactions pourrait fournir des outils de contrôle à l'industrie aquicole afin d'éliminer ou de restreindre ce prédateur. Sur le terrain, la densité et le taux de prédation de *C. lacteus* furent évalués en relation avec plusieurs types de perturbations sédimentaires. Ces outils de contrôle se sont tous montrées inefficaces. Les travaux de laboratoire ont confirmé l'impact de *C. lacteus* sur les populations de *M. arenaria*. L'analyse de la sélection de taille des myes n'a pas démontré de préférence significative. Une série d'expériences fut également effectuée afin d'évaluer l'effet de l'addition du polychète *Nereis virens* sur l'interaction entre *C. lacteus* et *M. arenaria*. Aucun

impact fut noté sur le taux de prédation de *C. lacteus*, cependant, *N. virens* a montré un comportement de fuite en présence de celui-ci.

Introduction

Aquatic species have become important resources in today's industry for economic and nutritional reasons. Some of these species have been the subject of heavy exploitation and, in some cases, natural populations have declined dramatically due to over fishing (Boghen 1995). The threat of depletion of natural stocks of commercial species has forced the industry to underline the importance of aquaculture in maintaining and improving marine productivity to keep up with consumer demands. The soft-shell clam (*Mya arenaria*) is considered by the industry to be an important species for shellfish aquaculture diversification in Atlantic Canada (Brown and al. 1995). Studies are underway to develop effective rearing techniques for this species.

Soft-shell clam farmers in Prince Edward Island, Canada, have noted the presence of the nemertean *Cerebratulus lacteus* (milky ribbon worm) on some of their culture sites, along with what is thought to be evidence of predation by this nemertean(e.g. mucus type substance on clam after contact with *C. lacteus*). The biology of *C. lacteus*, particularly its predation behavior, however, is poorly known. There is some anecdotal information possibly linked to predatory behavior, such as swimming behavior (Coe 1943, McDermott 1976) and gregarious behavior (Rowell and Woo 1990). Coe (1943) reported the preferred prey of *C. lacteus* to be polychaetes. Later observations showed that *C. lacteus* also preys on bivalves (McDermott 1976, Kalin 1984). After a massive mortality of soft-shell clams in Nova Scotia in 1987, Prouse and al. (1988) showed the importance of the presence of *C. lacteus* in the soft-shell clam habitat. A follow up study confirmed that *C. lacteus* is a predator of soft-shell clam, and that they may, in fact, be its preferred prey (Rowell and Woo 1990).

As clam farming grows in intensity, so does concerns about the potential for controlling an equal intensification of nemertean predation. Improved understanding of the interspecific relationship between *C. lacteus* and *M. arenaria* is therefore required in order to determine how to optimize predator control. The aim of this study is to gather informations on the impact of

habitat modification and interspecific relationships in order to develop control measures via 1) a field experiment to evaluate the impact of bottom manipulation through shell material addition and bottom cultivation (hydraulic rake) and 2) through laboratory experiments to determine interspecific relationships between two endobenthic worms (*C. lacteus* and *Nereis virens*) commonly encountered by the clam farmers and the soft-shell clams. The addition of shells may reduce the chance of predation by camouflaging the prey as well as to increase wound risks to the predator. *N. virens* were incorporated into the design to explore the possibility of using this species as a control measure since fisherman have noted a negative correlation between it and *C. lacteus*.

Material and methods

Field study

A field study was initiated on 8 July 1998 at Darnley Basin, Prince Edward Island (Fig. 1). Two sediment manipulations were carried out on a natural clam bed to evaluate their effect on the abundance of *C. lacteus* and their predation rate. Nine 3 m x 3 m plots were set up in the subtidal zone, marked with buoys. Each plot was sampled with a Venturi sampling system for a field inventory of the endobenthic macrofauna. Samples had a surface of 0.11 m^2 and about 0.2 m in depth (0.022 m^3). Three treatments, all triplicated, were applied randomly to entire plots. The three treatments were: 1) cultivation (hydraulic rake) 2) cultivation plus shell addition (clam shell fragments added to sediments) and 3) no manipulation controls. In the shelled treatment, about 0.32 m^3 of crushed shells were mixed to the sediments. In both the cultivated and shelled treatments, the hydraulic rake was used. The plots were left undisturbed until 22 September 1998, when each plot was sampled again. All macrofauna were weighed (fresh dry weight) and bivalves measured (length).

Laboratory studies

1) Predation

Laboratory studies were undertaken at Ellerslie Hatchery (Holland College), Prince Edward Island, on 29 June 1998, to study the relationship between *M. arenaria*, *C. lacteus* and *N. virens*. These three species are generally found in the same environment but with variable densities. All possible combinations were studied to observe the interspecific behavior. A total of 21 aquaria were used in this experiment. There were two sizes of aquaria: nine small (30 cm x 15 cm) and twelve large (38 cm x 25 cm). Sediments were sieved with a 1 mm mesh at Darnley Basin and transported to the laboratory. A layer of sediments approximately 10 cm deep was placed in each of the aquaria. The aquaria were supplied with salt water via a parallel flow-through system. The water was pumped from Bideford Estuary via a sand filter. The mean temperature was maintained at 21°C and mean salinity at 20‰. The flow of water pumped into the aquaria was adjusted with small clamps. The aquaria were then placed in larger plastic containers which served as a collecting bin for water overflow and any escaping organisms. The smaller aquaria were placed in pairs in the larger containers, whereas the larger aquaria were placed singly. A hole was cut out of the bottom of the plastic bin to serve as a drain. A rubber stopper with an elevated drain tube was inserted into the hole. The extremity of the tube was covered with a 2 mm mesh to prevent organisms from escaping. PVC tubing with a 15 cm diameter was used to support all of the plastic containers and serve as a drainway (Fig. 2). Photoperiod was maintained at 8 hours of light and 16 hours of darkness. The aquaria were left untouched for a week to let the sediment settle. The organisms were introduced into the aquaria on 7 July. There were seven different species combinations which were: *C. lacteus*, *M. arenaria* and *N. virens* (C/M/N); *C. lacteus* and *M. arenaria* (C/M); *C. lacteus* and *N. virens* (C/N); *M. arenaria* and *N. virens* (M/N); *M. arenaria* only (M); *N. virens* only (N) and *C. lacteus* only (C). *M. arenaria* and *C. lacteus* were obtained from West River, P.E.I. (Fig. 1), because of high population densities. The larger aquaria contained all combinations with *C. lacteus* and the smaller aquaria contained all other combinations. *N. virens* were obtained from Darnley Basin

also due to high population densities. The environmental conditions at these two locations were similar at the time of collection (T = 20°C and S = 27‰). The mean weight and standard deviation of *N. virens* used were 1.09±1.01g. Aquarium densities were established to resemble densities found on culture sites and fresh dry weight was taken before the organisms were introduced into the aquaria.. See Table 1 for total number of individuals per aquarium. The soft-shell clams were 20-25 mm in length. The aquaria were checked regularly for escaping organisms which were recorded and weighed. On the 5th of August (30 days after the introduction of organisms) the aquaria were emptied into a sieve and all organisms collected were weighed and their condition recorded.

2) Clam size selection

Another set of aquaria were set up on the 17th of August to determine the preferred clam size of *C. lacteus*. The addition of crushed clam shells and *N. virens* were also tested to evaluate their effect on the predation rate of *C. lacteus*. The set up was identical to the predation experiment, however, there were only 15 large aquaria (38 cm x 25 cm) used. Five treatments, all triplicated, were tested: *M. arenaria* only as a control (M); *C. lacteus* and *M. arenaria* without shells (C/M (ns)); *C. lacteus* and *M. arenaria* with shells (C/M (s)); *C. lacteus*, *M. arenaria* and *N. virens* (C/M/N) and *C. lacteus* and *N. virens* (C/N). The shells used were soft-shell clam pieces without any flesh. About 35% of the volume of each aquarium was occupied by clam shell. The sediments were left for a week before the introduction of the test organisms on the 24th of August. The soft-shell clams and *N. virens* were obtained from Darnley Basin due to population abundances and size range available. Individual *N.virens* were significantly larger, 5.09±0.41g (mean±SE), than in the experiment on predation. Their mean weight and standard deviation was. *C. lacteus* were obtained from West River. Four size-classes of clams were used: 0-15 mm; 15-35 mm; 35-50 mm and 50+ mm in length. Five clams from each size-class were used for a total of 20 individuals per aquarium. One *C. lacteus* was used per aquarium in order to reduce the predation rate observed in the first experiment. Ten *N. virens* were installed per aquarium in the C/N and C/M/N treatments to evaluate their effect on the predation rate of *C.*

lacteus. The aquaria were checked daily and any escaping organisms were recorded and weighed. Clams were weighed and condition noted once a 50% mortality was attained (17 September). Clam mortality was monitored by counting active siphons at the sediment surface. Aquaria were emptied into a sieve to retrieve all the test organisms.

Statistical Analysis

The data were analyzed with Systat 6.0© for Windows©. All analysis were carried out at α level of 0.05. The field data were analyzed with single factor ANOVAs. Plots were analyzed before treatments were applied to see if diversity and abundance were homogeneous. The plots were also analyzed after treatments were applied in relation to 4 variables: 1) diversity (total number of species); 2) total species abundance; 3) abundance of *M. arenaria*; 4) abundance of *N. virens*.

Laboratory work on predation did not require statistical analysis to evaluate the mortality of *M. arenaria* in relation to the different species combinations since there was an obvious variation between treatments (100% vs. 0%). The effect of the addition of *N. virens* was analyzed with single factor ANOVAs. The independent variable was species combinations. The dependent variables tested were: 1) the migration of *N. virens*; 2) the mortality of *N. virens*.

The laboratory data on prey size selection was analyzed with a two-factor ANOVA. The factors involved were size-class and sediment treatment. The data was transformed using the Arcsine transformation to assure homoscedasticity. Analysis of the absolute difference of weight in *C. lacteus* in relation to the treatments was accomplished with a single-factor ANOVA.

Results

Field study

The macrofauna (diversity; total species abundance; abundance of *M. arenaria* and abundance of *N. virens*) appeared homogenous throughout the plots. The statistical analysis showed no significant differences in plots before treatments were applied for the variables that were tested ($P > 0.05$). No significant difference was observed after treatments were applied for the same variables ($P > 0.05$). This indicates that the treatments had no effect on the species studied (Fig. 3). The abundance of *C. lacteus* and *N. virens* were very low in the study site and remained low despite the field manipulations.

Laboratory study

1) Predation

Our study showed 100% mortality of *M. arenaria* in all replicates of C/M/N and C/M treatments and 0% mortality in all replicates of M and M/N (Fig. 4). The addition of *N. virens* (C/M/N) did not decrease mortality of *M. arenaria*. Moreover, *N. virens* was observed migrating out of aquaria containing *C. lacteus* ($P=0.089$) (Fig. 5). The analysis of variance did not show any significant difference in migration of *N. virens* between treatments. *N. virens* that were not retrieved when the aquaria were emptied or as escapees were considered dead. Mortality of *N. virens* varied between 40% and 55% throughout the treatments (Fig. 5). These variations were not significant ($P=0.212$).

2) Clam size selection

C. lacteus showed no preferred size class of soft-shell clams ($P=0.879$). However, the 0-15 mm size class showed less mortality than the other size groups. The 15-35 mm and 35-50 mm

size classes showed high mortality compared to the control M, which showed no mortality. There was, however, a significant difference between M and the two treatments C/M/N and C/M(s) ($P=0.003$). No interaction between size-classes and experimental treatments was observed ($P=0.505$). No difference in mortality between the aquaria containing C/M/N and C/M(s) and the control aquaria C/M(ns) was observed. Although there is, however, a slight tendency of increased mortality in clams with the addition of shells and *N. virens* when compared to the control C/M(ns) (Fig. 6).

The analysis of the absolute difference in weight of *C. lacteus* showed no significant difference before and after treatments ($P = 0.324$). There was a positive correlation between weight of *C. lacteus* and the presence of clams (Fig. 7). The presence of *N. virens*, in the absence of clams, was not sufficient to allow any increase of weight by *C. lacteus*.

Discussion

Field study

The two control measures tested in the natural environment were found to be ineffective in reducing predation by *C. lacteus* on soft-shelled clams. However, field densities of *C. lacteus* were low inside plots, with only one individual per square meter. Rowell and Woo (1990) mentioned that field densities could reach 60 ind./m^2 in similar environments in Nova Scotia, which they attributed to the worms gregarious behavior. Rowell and Woo (1990) also showed that high densities of *C. lacteus* were associated with mortalities of clams. It is, therefore, possible that the *C. lacteus* densities were not large enough to be affected by sediment manipulations or affect predation rate. It would be advantageous to test these manipulations with higher densities of *C. lacteus*. These results, however, do suggest that the use of the mechanical harvester did not affect clam mortality levels or *C. lacteus* abundance, a point of great concern to some clam farmers.

Laboratory study

1) Predation

The laboratory study confirmed *C. lacteus* as an efficient predator of *M. arenaria*. The presence of *C. lacteus* was associated with the very high mortality (100%) of *M. arenaria* in the aquarium trials. This was confirmed by the total absence of clam mortality in the absence of *C. lacteus*. A second experiment showed low mortality in the control treatments. In 30 days, two nemerteans killed 18 clams which represents a fairly high predation rate. However, complete mortality was probably achieved before the end of the 30 day period since *C. lacteus* migrated out of the aquariums during the last week. Rowell and Woo (1990) showed a similar field migration of *C. lacteus* when mortality of a clam population was complete, thus, predation rate is likely underestimated. The study on the interspecific relationship between *C. lacteus* and *N. virens* revealed a negative response of *N. virens* to the presence of *C. lacteus*. Coe (1943) even observed *C. lacteus* swallowing a *Nereis* of about its own diameter. In the present study, mortality of *N. virens* did not increase when no other food sorce was available to *C. lacteus*. This suggests that *C. lacteus* does not prey upon *N. virens*, although there might be some territorial behavior between the two species.

2) Clam size selection

This study showed no preferred clam-size for *C. lacteus* predation. There is, however, a lower percentage of mortality in the 0-15 mm size-class (except when *N. virens* was present). The polychaete, *N. virens*, is known for its predation on juvenile soft-shell clams (Commito 1982), thus, the higher mortality observed in this study, could be attributed to the presence of *N. virens*. Moreover, there was a higher mortality of 0-15 mm size class in the absence of the nemertean. This indicates that nemertean predation on this size class could have been

overestimated. Predation was highest in 15-35 mm and 35-50 mm size classes, since no mortality was observed in the absence of the nemertean.

Analysis of weight of *C. lacteus* showed an increase in the presence of *M. arenaria* (≈2X). Conversly, a reduction in weight of *C. lacteus* in the presence of *N. virens*, suggests that there is no predation on *N. virens*. The migration rate of *N. virens* was lower in the presence of *C. lacteus* during the second experiment (prey size) compared to the 1st experiment on predation. Although the reason for this is not clear, it may be due to size of the individual nereids used.

Conclusion

Sediment manipulations were proven ineffective in reducing the predation rate of *C. lacteus* on the field at densities of $1/m^2$. This was reinforced by laboratory experiments. The addition of *N. virens* was also proven ineffective in providing a "decoy" prey for controlling predation on clams. In addition, *N. virens* was found to have a negative response to the presence of *C. lacteus*, although there was no indication of predation on *N. virens* by *C. lacteus*. The study on prey size selection showed no preference of any size-class, however, the 0-15 mm size-class seemed to be less favored than the 15-35 and 35-50 mm size-classes. These results should be confirmed through more prey size experiments.

These studies provide a preliminairy basis for further, detailed study of the milky ribbon worm and clam predation. Future studies will look at the predation mechanisms involved in the attack on soft-shell clams to develop effective control measures. For example, we need to know the time budget of the nemertean as well as whether the act of predation is endobenthic or epibenthic.

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Table 1: Species combinations and number of individuals in aquaria for a) predation experiments and b) clam size selection.

	Combination	Species	Number in aquaria
a)	C/M/N	*C. lacteus*	2
		M. arenaria	18
		N. virens	10
	C/M	*C. lacteus*	2
		M. arenaria	18
	C/N	*C. lacteus*	2
		N. virens	10
	M/N	*M. arenaria*	18
		N. virens	5
	M	*M. arenaria*	10
	N	*N. virens*	5
	C	*C. lacteus*	2
b)	M	*M. arenaria*	20
	C/M(ns) no shells	*C. lacteus*	1
		M. arenaria	20
	C/M(s) shells	*C. lacteus*	1
		M. arenaria	20
	C/M/N	*C. lacteus*	1
		M. arenaria	20
		N. virens	10
	C/N	*C. lacteus*	1
		N. virens	10

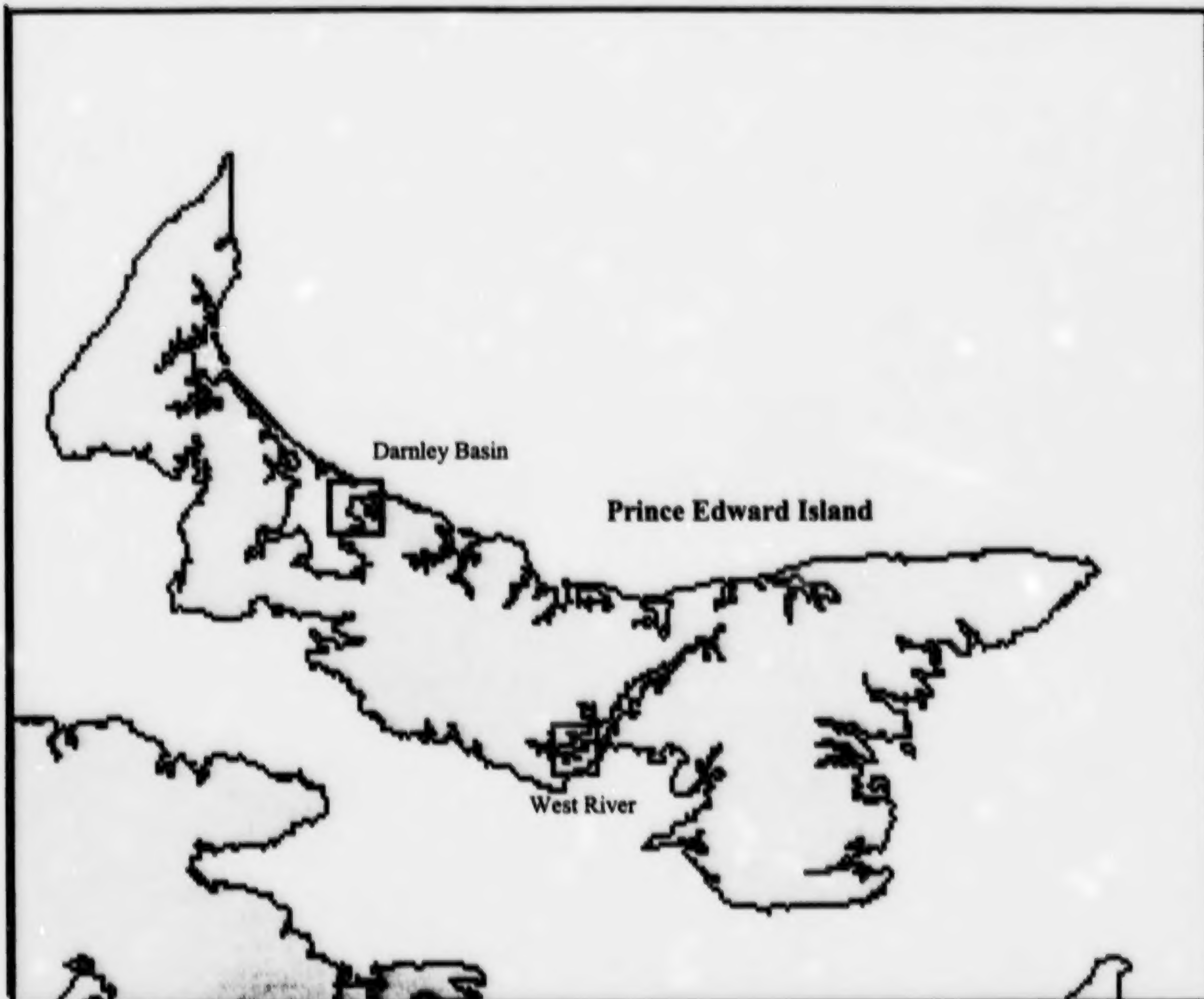

Figure 1: Location of Darnley Basin and West River in Prince Edward Island.

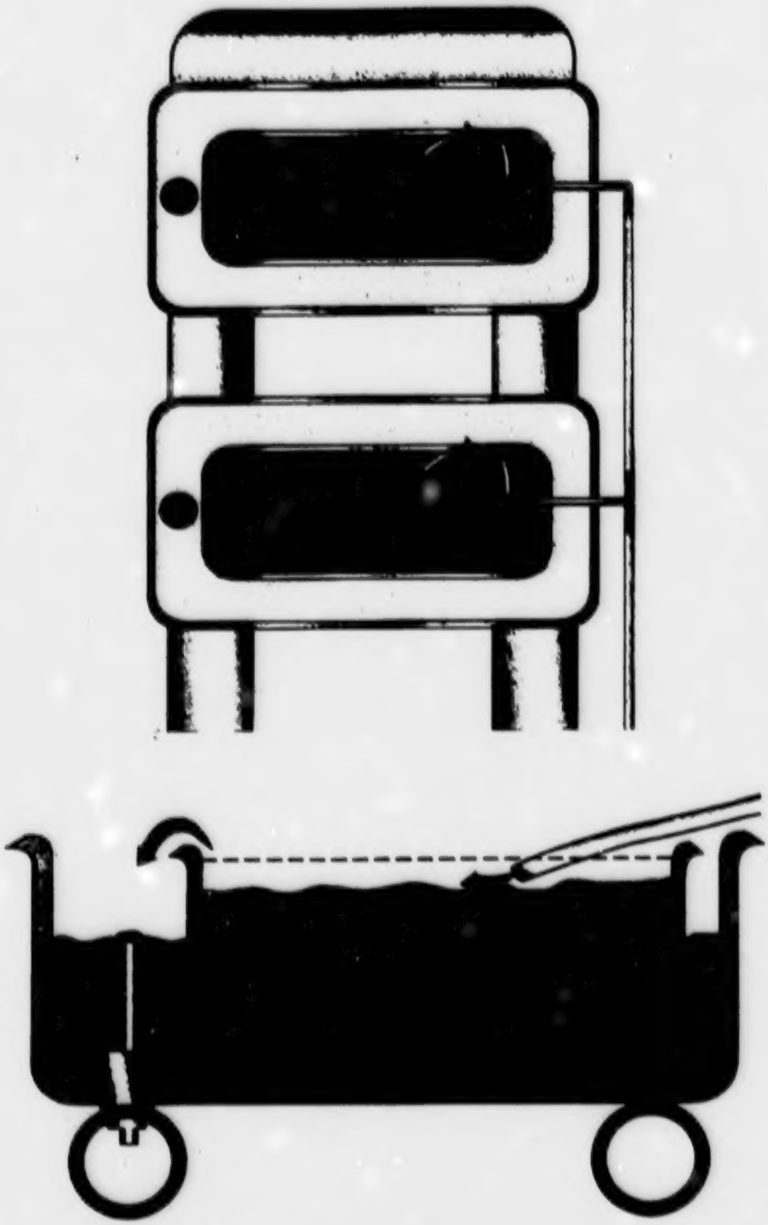

Figure 2: Experimental set-up in Ellerslie laboratory.

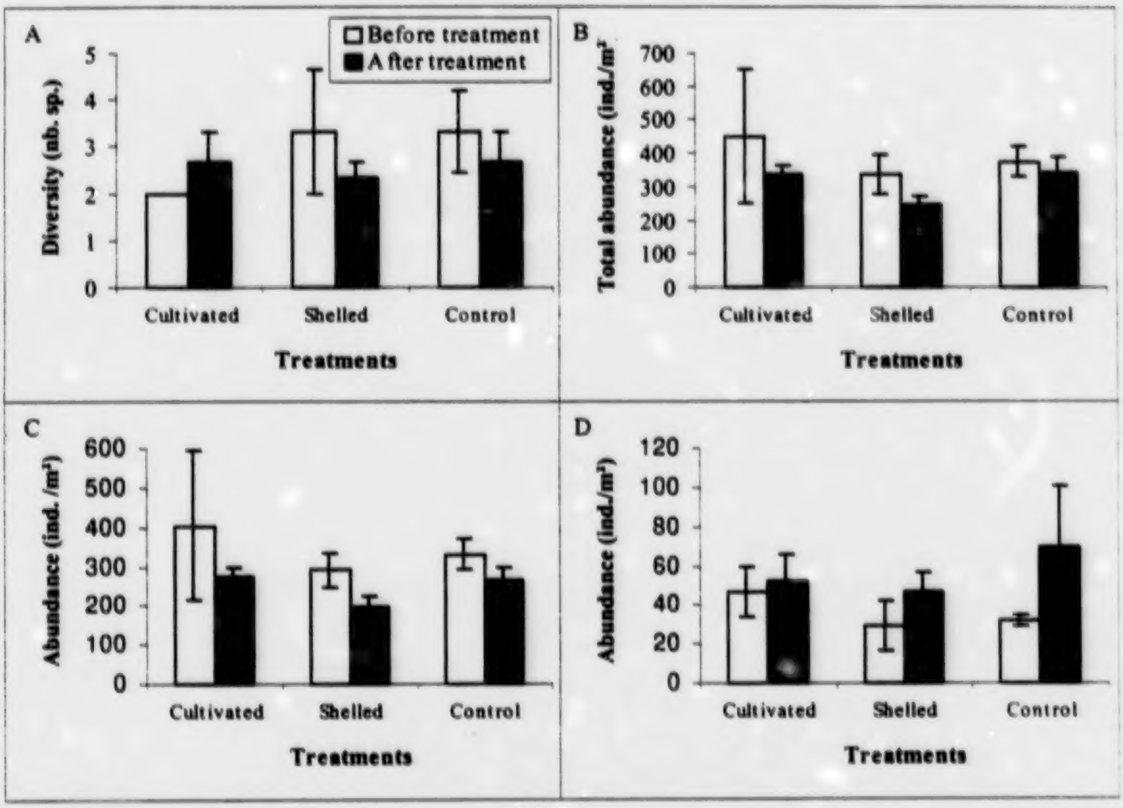

Figure 3: Description of the macrofauna before and after sediments were manipulated (cultivated and addition of shells) at Darnley Basin, P.E.I., for the following variables (mean ± SE): A) diversity (total number of species) B) total species abundance C) abundance of *Mya arenaria* and D) abundance of *Nereis virens*.

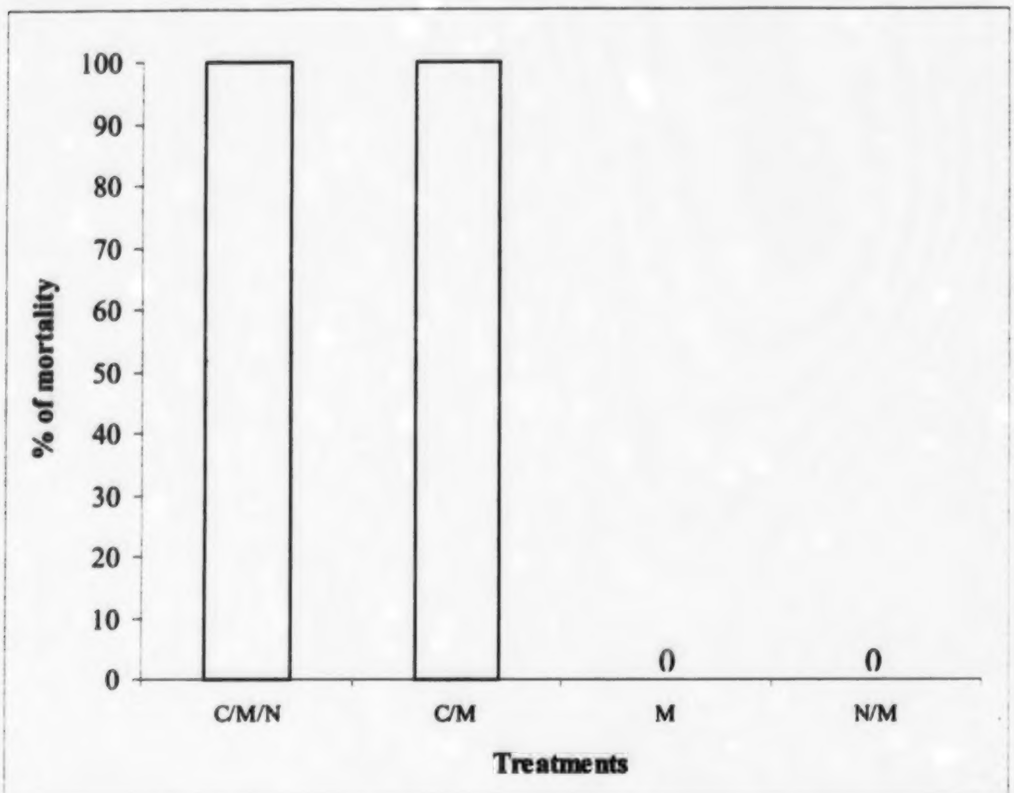

Figure 4: Percentage of mortality of *Mya arenaria* in relation to different species combinations (C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*; C/M: *C. lacteus* and *M. arenaria*; M: *M. arenaria*; N/M: *N. virens* and *M. arenaria*).

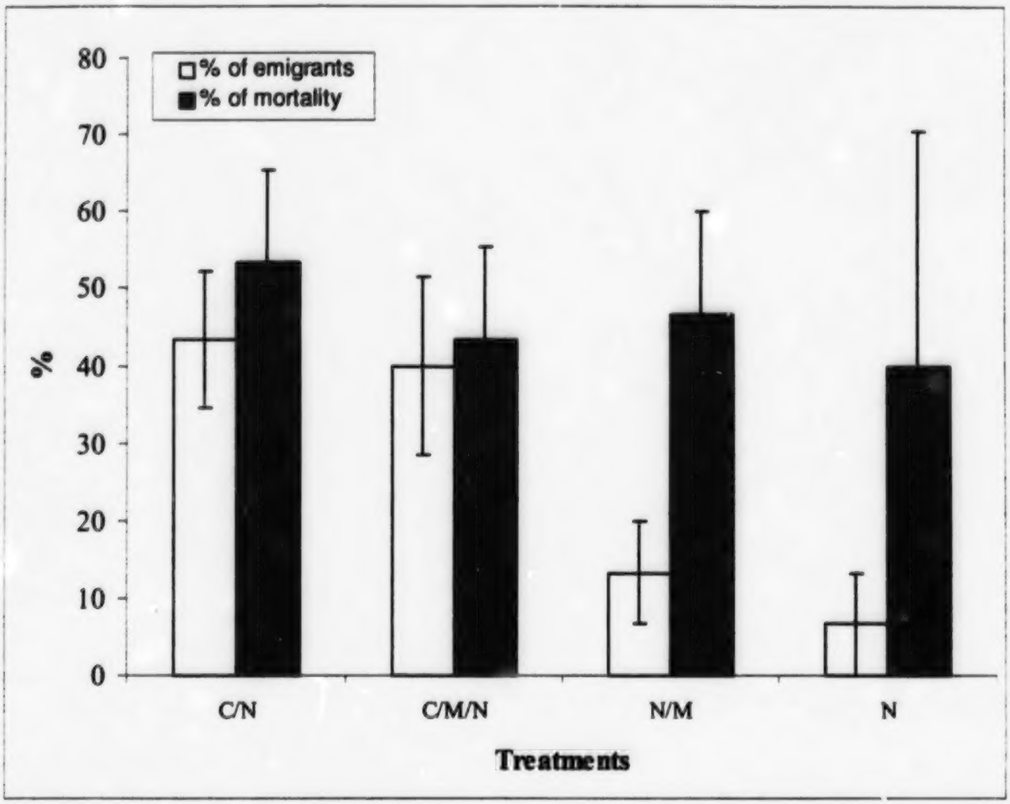

Figure 5: Percentage (mean ± SE) of migration and mortality of *Nereis virens* in relation to different species combinations (C/N: *C. lacteus* and *N. virens*; C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*; C/M: *C. lacteus* and *M. arenaria*; N/M: *N. virens* and *M. arenaria*; N: *N. virens*).

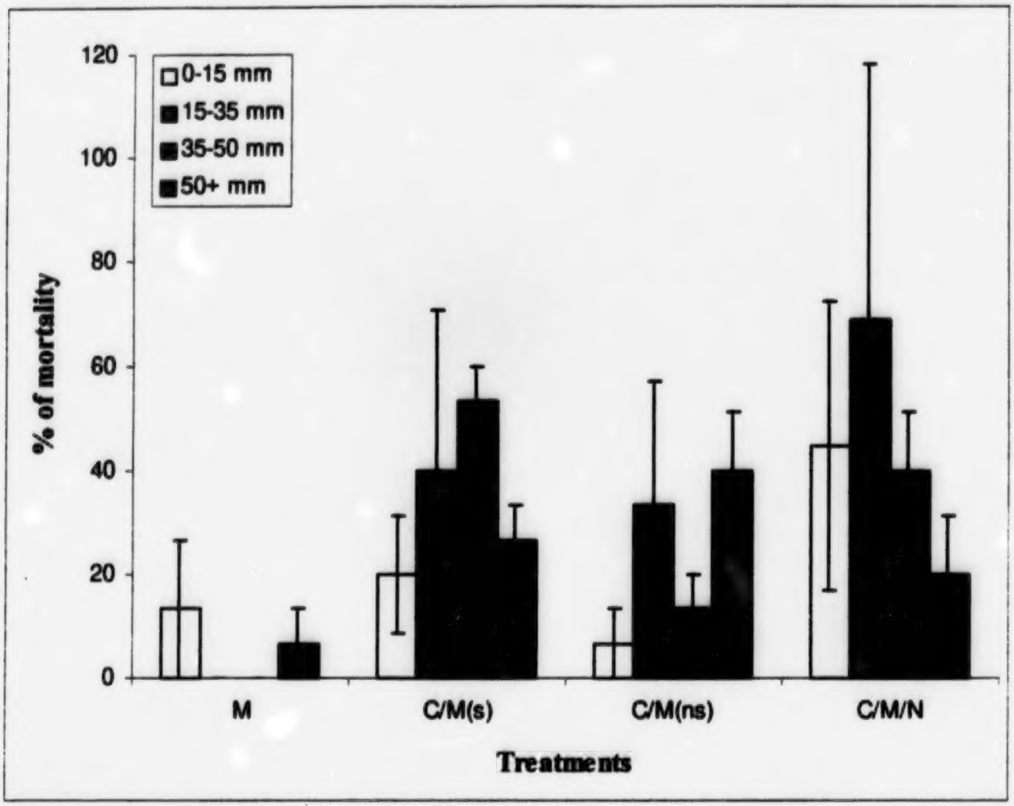

Figure 6: Percentage of mortality per size-class (mean±SE) of *Mya arenaria* in relation to treatments and species combinations (M: *M. arenaria*; C/M(s): *C. lacteus* and *M. arenaria* with shells; C/M(ns): *C. lacteus* and *M. arenaria* with no shells; C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*).

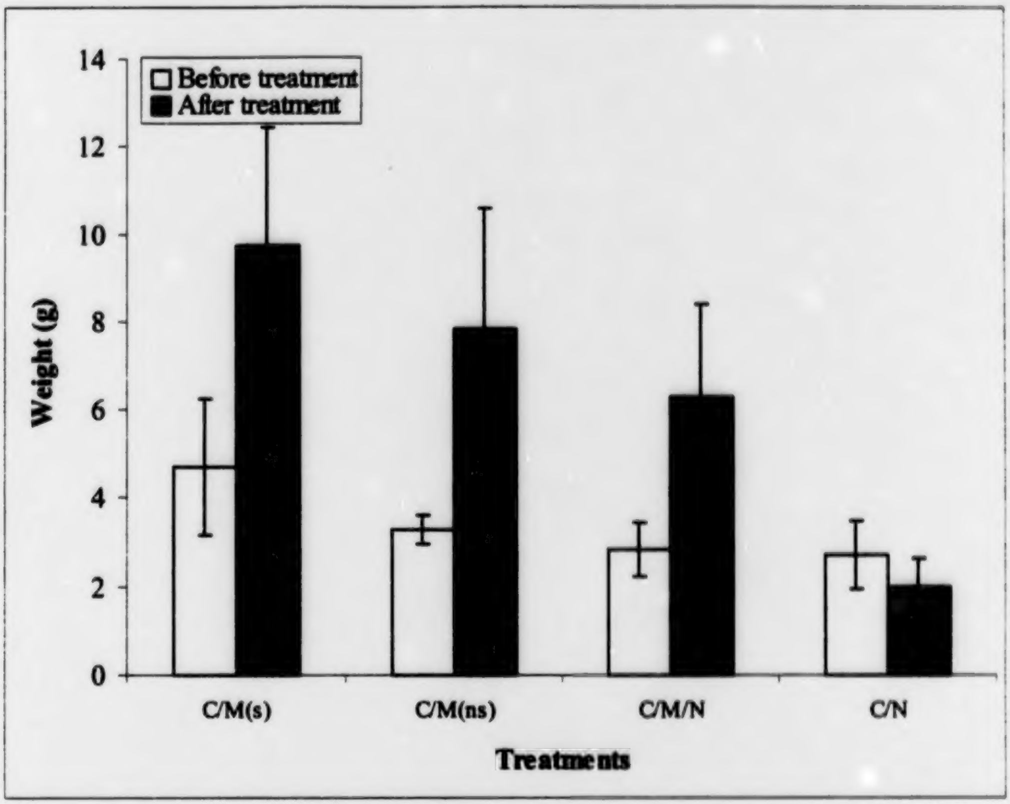

Figure 7: Weight (mean ± SE) of *Cerebratulus lacteus* before and after experimental treatments (C/M(s): *C. lacteus* and *M. arenaria* with shells; C/M(ns): *C. lacteus* and *M. arenaria* with no shells; C/M/N: *C. lacteus*, *M. arenaria* and *N. virens*; C/N: *C. lacteus* and *N. virens*).